new matter has been added by virtue of the amendments made to the claims.

Claim Rejections - 35 U.S.C. §112

The Examiner acknowledges on page 3 of the Office Action dated 10/21/02 that the Specification, is enabled for

> creating a DNA having the target single-stranded region within a double-stranded region by nicking at least two sites bordering the target region within the double-stranded DNA with at least one site-specific nicking endonuclease.

but has rejected claims 1 and 3-5 as not enabled asserting that

> a DNA having the target single-stranded region within a double-stranded region cannot be created after digestion of a double-stranded DNA with a site-specific nicking endonuclease and selectively denatured the double-stranded DNA if the double-stranded DNA only has one site for the site-specific nicking endonuclease.

Claim 1 has been amended and now requires nicking at two or more sites or nicking at one site and cleavage at a second site. Figure 1A shows how two nicks on one strand can generate a single stranded gap. Figure 1(B) (left hand) shows how two nicks on opposite strands can generate two molecules with single stranded tails containing the target single strand region.

Claim 3 has been amended so as to more specifically require that when the site specific nicking activity occurs near the terminus of the double-stranded DNA, a single stranded region can be generated at the terminus of the DNA. For reasons of clarity, claim 3 has been placed in independent form. According to the method of claim 3, a

single nick can create the desired target single-stranded region as illustrated in Figure 1B (right side) when the target single strand region is created at the terminus of a double stranded linear molecule. Further discussion of such situations are given on page 15, lines 2-7 of the Application.

> **...Alternatively, single-stranded termini could be created by introduction of a single nick near the terminus of a linear molecule, where the linear molecule either exists naturally, is a synthetic molecule, or is created by *in vitro* reactions such as, but not limited to, restriction endonuclease cleavage or the polymerase chain reaction (PCR).**

The Figures and description in the Application instruct one skilled in the art how to use a single nicking event to create single-stranded DNA at double stranded termini of linear molecules by virtue of a single nicking event occurring on one strand of the DNA duplex bordering that terminal target region.

Claims 4 and 5 further describe how a linear molecule is the substrate for the nicking reaction (Claim 4) or such linear molecule is created by restriction endonuclease cleavage (Claim 5).

Claim 2 is rejected under 35 U.S.C. ¶112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicants respectfully traverse the rejection, because prior to amendment, claim 1 required at least one nicking site within the double-stranded DNA for site-specific nicking endonuclease while claim 2 required at least two nicking sites within the double-stranded DNA for site-specific nicking endonuclease and "at least two nicking sites" is

a subset of "at least one nicking site". However, Applicants have amended claim 1 and 2 to advance prosecution and it is submitted that the Examiner's rejection is now moot.

In view of the amendments to claims 1-5, the Examiner is respectfully requested to reverse the above rejections. Applicants reserve the right to prosecute additional subject matter relating to claims prior to amendment to be prosecuted in a Divisional Application.

Claim Rejections - 35 U.S.C. §102

Claims 30-34 are rejected under 35 U.S.C. §102(b) as being anticipated by Xu, et al. (U.S. Patent No. 5,786,195, published on July 28, 1998). Applicants respectfully traverse the Examiner's rejection. Claims 30-34 are canceled although Applicants reserve the right to submit the canceled claims in a Divisional Application. The rejection is now moot.

Claims 1, 33 and 34 are rejected under 35 U.S.C. §102(a) as being anticipated by Wang, et al. (*Molecular Biotechnology*, 15:97-104, June 2000).

Below, Applicants explain why Wang, et al. does not teach the present claimed method as defined in claim 1. Claims 33 and 34 are canceled.

Paragraph 10 (page 7 of the office action dated 10/21/02) rejects Claim 1 as being anticipated by Wang *et al.*, (*Molecular*

Biotechnology, 15:97-104, June, 2000). In this reference, two vectors differing by a single nucleotide, were denatured and reannealed to give a hybrid molecule with a single base mismatch and a nick in one strand of the circular molecule. The Examiner has suggested that the mismatch can be considered both as a target region and as a single-stranded region. Even if the mismatch site is so considered, the molecule described by Wang, *et al.* differs significantly from those claimed in this Application. No site-specific nicking occurs on the border of the target region. Wang et al state:

> "...pUC19XE [is used] to construct a DNA substrate containing a G/T mismatch and a single nick in the antisense strand 130 bases 3' to the mismatch ...." (page 98).

Having a nicking site some 130 bp distant from the target site can hardly be considered as a"...site bordering the target region..." as recited in Claim 1 and described in the above application. In the experiments of Wang, *et al.*, the nick does not define the target region, rather it is useful only as a topological tool, allowing for the formation of a circular heteroduplex of DNA.

Claim 1 element (a) as amended requires

> nicking at least two sites bordering the target region within the double-stranded DNA with at least one site-specific endonuclease

Wang et al. does not therefore teach the method claimed in claim 1 nor is there any suggestion to use nicking enzymes as claimed.

For the reasons set forth above, Applicants respectfully submit that the rejections set forth in the Official Action of October 21, 2002

have been overcome and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made herein, the undersigned Attorney would appreciate the opportunity to do so. Thus, the Examiner is hereby authorized to call the undersigned, collect, as the number shown below.

Respectfully submitted,

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Date: 2/11/03

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MARKED-UP VERSION OF THE CLAIMS

1. (amended) A method for creating a target single-stranded region in a double-stranded DNA, comprising:
(a) nicking at least [one site] <u>two sites</u> bordering the target region within the double-stranded DNA with at least one site-specific nicking endonuclease; and
<u>(b)</u> [(c)] subjecting the nicking DNA to conditions where the target region is selectively denatured [; to create the target single-stranded region in the double-stranded region].

3. The method [of claim 1 wherein the] <u>for creating a</u> target single-stranded region [comprises at least one] <u>at a</u> terminus [in the] <u>of a linear</u> double-stranded DNA [and wherein said method] comprises:
(a) nicking at least one site bordering the target region [in a first strand of] the double-stranded DNA with at least one site-specific nicking endonuclease, [wherein the second strand of the double-stranded DNA has at least one break bordering the target region]; and
(b) subjecting the resulting DNA to conditions where the target region is selectively denatured.

4. The method of claim 3 wherein the [break in the second strand] <u>DNA terminus</u> is pre-existing.

5. The method of claim 3 wherein the [break in the second strand is produced by a] <u>DNA terminus is formed by</u> site-specific endonuclease <u>cleavage</u>.